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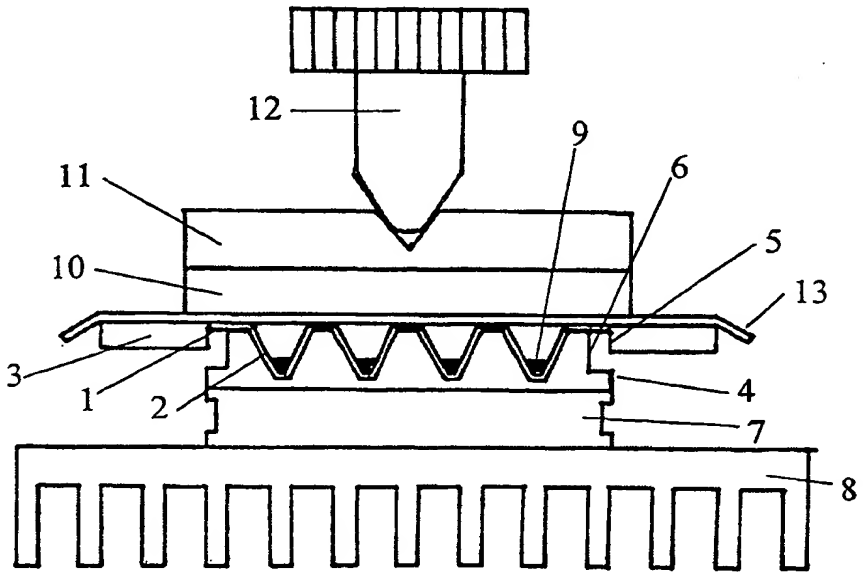

(57) Abstract

Ultrathin-walled multiwell reactors for heat block thermocycling of samples comprising an array of small-volume wells of identical height with similarly shaped sample wells formed in the top surface of the heat block of the thermocycler are provided. The multiwell plates are preferentially vacuumformed out of a 30-50 micron thick thermoplastic film and can be used for rapid, oil-free temperature cycling of small (1-10μl) volume samples.

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Ultrathin-walled multiwell plate for heat block thermocycling

The invention relates to plastic plates for conventional heat block thermocycling of biological samples, particularly to multiwell plates. More specifically, it relates to ultrathin-walled multiwell plates with an improved heat transfer to small-volume samples. Such plates can be used for rapid temperature cycling of multiple, small-volume samples (i.e. 1-20 µl) by using heat block thermocyclers with an increased block temperature ramping rate (i.e. 4 ° C/second and greater) and standard heated-lid technology for sealing the samples.

Temperature cycling of biological samples is a central moment in DNA amplification by the polymerase chain reaction (PCR) (Saiki et al., Science, 239, 487-491 [1988]). Much effort is being expended in developing various alternative reactors and technologies for rapid temperature cycling of small-volume samples (Kopp et al., Science 280, 1046-1048 [1998]; Belgrader et al., J.Forensic Science 43, 315-319 [1998]; Wittwer et al., Analytical Biochem., 186, 328-331 [1990] and U.S. Patent No 5,455,175; Woolley et al., Analytical Chem., 68, 4081-4086 [(1996]).

One commercially available type of microreactor and thermocycler for rapid temperature cycling of small samples is a glass capillary tube and a hot-air thermocycler from Roche Molecular Biochemicals (cat No. 1909 339 and cat No. 2011468, respectively). The glass capillary tube can hold reaction volumes ranging from 10 to 20 µl. The hot-air thermocycler can hold 32 capillaries and perform 30 - 40 PCR cycles in 20-30 minutes. However, these rapid DNA amplification technology is connected with various disadvantages, for example:

a) The handling of the individual capillaries is relatively cumbersome.

b) The relatively large glass surface adsorbs components of the standard PCR-mixtures. This might inactivate the reaction. Therefore, various carrier molecules, i.e. proteins or even DNA, must be added and the concentrations of the components reoptimized.

c) The cost of the capillary tube, as a disposable PCR container, is high when compared to the standard 0.2 ml PCR tube.

d) The experimental throughput using this system is limited.

It is surprising that only little research has been conducted to improve the basic performance in sample size and speed of the widely used, conventional heat block

thermocycling of samples contained in plastic tubes or multiwell plates. One known improvement of heat block temperature cycling of samples contained in plastic tubes has been described by Half et al. (Biotechniques, 10, 106-112, [1991] and U.S. Patent No 5,475,610). They describe a special PCR reaction-compatible one-piece plastic microcentrifuge tube, i.e. a thin-walled PCR tube. The tube has a cylindrically shaped upper wall section, a relatively thin (i.e. approximately 0.3 mm) conically-shaped lower wall section and a dome-shaped bottom. The samples as small as 20 µl are placed into the tubes, the tubes are closed by deformable, gas-tight caps and positioned into similarly shaped conical wells machined in the body of the heat block. The heated cover compresses each cap and forces each tube down firmly into its own well. The heated platen (i.e. heated lid) serves several goals by supplying the appropriate pressure to the caps of the tubes: it maintains the conically shaped walls in close thermal contact with the body of the block; it prevents the opening of the caps by increased air pressure arising in the tubes at elevated temperatures. In addition, it maintains the parts of the tubes that project above the top surface of the block at 95° -100° C in order to prevent water condensation and sample loss in the course of thermocycling. This made it possible to exclude the placing of mineral oil or glycerol into the wells of the block in order to improve the heat transfer to the tubes and the overlaying of the samples by mineral oil that prevented evaporation but also served as added thermal mass. In addition, the PCR tubes can be put in a two-piece holder (US patent 5,710,381) of an 8x12, 96-well microplate format, which can be used to support the high sample throughput needs with any number between 1 and 96 individual reaction tubes.

In DE 4022792 the inventors describe a plate with cylindrically shaped walls of the wells and spherically shaped bottoms thereof. The individual wells of the plate were formed by melting a polycarbonate sheet in the range of 0.27-0.5 mm by a stream of hot air. This technology leads to relatively thin walls in the range of 0.08-0.2mm. The biological samples were placed into the wells, covered with polycarbonate film (0.1 mm) and the individual wells were thermosealed by a special press. Upon sealing the plate was placed on the thermoblock and fixed by screws. Though theoretically the heat transfer to the samples is improved, however, the way of positioning the plate on the block and the cylindrical and spherical geometry of the well prevent a close thermal contact with the heating block. During thermocyling, due to the large thermal expansion, the plate fixed by

screws becomes deformed and the close thermal contact is not maintained anymore. Therefore, by using the above technology rapid cycling reactions cannot be performed.

The other known improvement of heat block thermocycling is described in PCT patent application WO 98/43740. It concerns a heat block thermocycler with an increased ramping rate, i.e. 4° C/second). The thermocycler can hold 96 PCR tubes (each of a volume of 0.2 ml) or 96-well PCR plates. Theoretically, the thermocycler can perform 30 PCR cycles in 20-30 minutes, provided that only a few seconds are spent to reach the temperature equilibrium between the heat block and the samples.

However, as described in U.S. Patent No 5,508,197, even if the temperature of the heat-transfer media, i.e. water, is changed almost instantaneously, it takes approximately 15 seconds to reach equilibrium between water and the 15-20 µl samples in the standard PCR plates. This means that for 30 PCR cycles approximately 20 minutes are spent to reach the equilibrium between heat-transfer media and the 15-20 µl samples in the plates.

In comparison, the above mentioned heat block cycler (WO 98/43740) operating at a ramping rate of 4° C/second, needs for the heat-block temperature transitions during 30 PCR cycles 10 minutes only. This shows that the major limiting factor for rapid temperature cycling of small samples in platic PCR tubes or PCR plates is the low efficiency of the heat transfer through the walls of conventional PCR tubes or plates, respectively.

The present invention concerns plastic multiwell plates for performing heat block thermocycling of multiple samples. More specifically, it concerns ultrathin-walled multiwell plates with an improved heat transfer to small samples. Ultrathin-walled multiwell plates are suited for rapid, oil-free, heat block temperature cycling of small-volume samples (i.e. approximately 1-20 µl), whereas the lower limit is given by the reliability of the conventional pipetting systems.

Figure 1 illustrates an example of a multiwell plate according to the invention.

Figure 2 illustrates the positioning of the plate in the block of the thermal cycler.

One aspect of the present invention concerns the considerably decreased thickness (i.e. approximately 7.5-15 fold) of the well walls when compared to known thin-walled PCR tubes (U.S. Patent No 5,475,610). This can be reached, for example, by means of thermoforming the plates out of thin thermoplastic films. Such thermoplastic films are, for example, polyolefin films, such as metallocene-catalyzed polyolefin films and/or copolymer films. Usually, the multiwell plate is vacuumformed out of cast, unoriented

polypropylene film, polypropylene-polyethylene copolymer films or metallocene-catalyzed polypropylene films. The film is formed into a negative ("female") mould comprising a plurality of spaced-apart, conically shaped wells which are machined in the body of a mould in the shape of rectangular- or square-array. The thickness of the film for vacuumforming conically shaped wells is chosen according to the standard rule used for thermoforming, i.e. thickness of the film = well draw ratio x thickness of the wall of the formed well.

For example, vacuumforming wells with a draw ratio of two and an average thickness of the walls of 30 microns results in a film thickness of 60 microns. The average optimum wall thickness was found to be 20-40 microns. The thickness of the well is reduced 7.5-15 fold when compared to the wall thickness of the formerly improved PCR tube desribed in U.S. Patent No 5,475,610. Using the Fourier equation for heat transfer and the equation for temperature transfer through solid substances, it can be shown that heat transfer through one square millimeter of the surface of the well of the plate is increased 7.5-15 fold and the time of temperature transfer through the wall is decreased 56-225 fold when compared to the said PCR tube. This drastic decrease in time can be explained by the fact that the time needed for the transfer of temperature front is proportional to the square power of distance. It can be easily calculated that the time of the temperature transfer through the ultrathin walls of the multi-well plate is in the range of milliseconds, whereas for the said PCR tube (U.S. Patent No 5,475,610) it is in the range of seconds. This explains the well known fact that thin (20-40 microns) plastic films are poor thermo insulators.

The thickness of the walls of the formed wells is gradually reduced to the bottoms of the wells due to vacuumforming of the wells into a negative mould. This geometry of the walls of the wells provides several advantages:

- The relatively thick upper parts of the walls of the wells cause additional rigidity of the whole multiwell plate.
- During heating of the heat block of the thermocycler, a vertical temperature gradient is formed in the sample, due to the gradient of the well-wall thickness. This vertical temperature gradient causes intensive convective mixing of the sample in conically shaped wells and increases the heat transfer through the sample. In comparison, this convective mixing of the sample is much less efficient in conventional PCR plates/tubes with a uniform wall thickness.

Another aspect of the invention concerns the height of the wells of the multiwell plate. The height of the conically shaped wells is equal to the height of the similarly shaped sample wells machined in the body of the heat block. Thus, this geometry of the wells (2) enables the positioning of the plate (1) on the heat block (4) as shown in Figure 2. As shown (Figure 2), in contrast to the conventional PCR plates, the walls of the wells (2) of the multi-well plate (1) do not project above the top surface of the block (4). The type of positioning provides several advantages: The pressure caused by the screw (12) to the lid (10) (heating element (11)) can be increased in order to obtain efficient sealing of the samples (9) sealed, for example, by a silicon mat (13). In this case the pressure is actually directed to those parts of the multiwell plate (1) which are supported by the top surface of the heat block (4) (or by parts of the top surface surrounding individual wells depending on the geometry of the heat block) and not to the thin walls of the wells of the plate as it is the case for the PCR tubes or conventional PCR plates. This advantage makes it possibe to increase the sealing pressure of the heated lid (10) several fold when compared to the conventionally used pressure of 30-50 g per well without cracking the conically shaped walls of the wells (2).

The extremely thin walls of the wells, i.e. 20-40 microns, are highly flexible as the multiwell plates are thermoformed out of highly elastic films (or sheets depending on the draw ratio). The walls of the wells are highly resistant against stress cracking, due to their flexibility and elasticity. As the wells of the plate, positioned on the heat block, are tightly sealed at room temperature, the air pressure in the wells will increase at elevated temperatures. The increased air pressure causes a deformation of the walls of wells and brings them in tight thermal contact with the surface of the walls of the individual sample wells machined in the body of the heat block. Standard PCR plates (having relatively thick and rigid walls of the wells) require that the conically shaped walls of the wells have to match perfectly with the shape of the wells machined in the body of the heat block to guarantee a close thermal contact (see for example U.S. Patent No 5,475,610). This requirement is not as critical for the ultrathin walled multiwell plates of the invention, due to flexibility and elasticity of the walls of the wells. Using this advantage, special shapes of both, the walls of the wells of the plate and the wells of the heat block can be differently designed. These differently designed wells can promote an even closer thermal contact after positioning the plate into the heat block.

Another aspect of the invention concerns the frame of the multiwell plates. As the plates can be formed of very thin films (depending on the draw ratio of the well; supra) the flexibility of, for example, standard-format plates, i.e. 96-well PCR (8,5 x 12,5 cm) plates, is such that handling is not easily possible anymore. Therefore, depending on the geometry of the plate, a supporting frame might be needed, for example for industry standard formats, i.e. 96-, 192-, 384-well PCR plates. This frame can support, for example in case of small plates, the edges of the plate, or individual wells of the plate, or groups of wells. For handling with robots, for example, the frame can be injection molded in the form of the standard skirted microplates containing the array of holes in the top surface of the frame matching the array of wells of the ultrathin multiwell plate. The plate can be attached to the frame by for example heat bonding. However, for small format plates including the frame can be formed as a single piece by using specially designed moulds.

The polypropylene-based plastics are PCR-compatible and therefore widely used for injection molding of PCR tubes and/or multiwell plates. In addition, they are resistant to stress cracking and have a reduced water vapor sorption when compared to other plastics (e.g. polycarbonate). Such plates can be thermoformed in both, standard industry formats, i.e. 96-, 192- and 384-well PCR plates for large scale applications, supported by robots and small foot-print formats to match small foot-print thermocyclers, i.e. "personal thermocyclers".

The following example serves to illustrate the invention but should not be construed as a limitation thereof.

Example:

Fig.1 illustrates a 36-well ultrathin walled multiwell plate according to the invention. The plate was designed for rapid temperature cycling of samples ranging from 0.5-4 µl using a small foot-print peltier-driven heat block thermocycler supplied with a "wine-press" type heated lid (Fig. 2). The volume of the wells is 16 µl and the distance between the wells is 4.5 mm, i.e. industry standard for high sample density 384-well PCR plates. The diameter of the openings of the wells is 3.8 mm and the height of the wells is 3 mm. The average thickness of the walls of the wells is 30 µm. The frame (3) was cut out of a polypropylene sheet of a thickness of 0.5 mm and heat bonded to the plate (1). The area of the plate (1)

is 30 x 30 mm. As shown in Figure 1, the handling of the plate (1) containing the multiple wells (2) is facilitated, by a rigid 0.5-1 mm thick plastic frame (3) which is heat bonded to the plate. As shown in Figure 2, the frame (3) is not in direct thermal contact with the block (4) during thermocycling because the inner contour (5) of the frame (3) matches the outer contour (6) of the heat block (4) of the thermocycler (7 = thermoelectric heat pump and 8 = air-forced heat sink).

The ultrathin walled multiwell plate according to the invention (Fig.1) was experimentally tested for the amplification of a 455-base pairs long fragment of human papilloma virus DNA. The sample volume was 3 µl. For various PCR reactions, the average ramping rate of the thermo cycler was varied from 4° C to 8° C per second. The samples (i.e. standard PCR-mixtures without any carrier molecules) were transferred into the wells of the plate by means of conventional pipetting equipment. The plate was covered by standard sealing film (Microseal A; MJ-Research, USA), transferred into the heatblock of the thermocycler and tightly sealed by the heated lid as shown in Fig. 2. Upon sealing, a number of 30 PCR cycles was performed in 15-25 minutes depending on the ramping rate of the thermo cycler. The PCR product was analyzed by conventional agarose electrophoresis. The 455-base pairs long DNA fragment was amplified with a high specificity at the indicated ramping rates (supra).

Plates according to the invention with well volumes of 35 µl were successfully tested for temperature cycling of samples of a volume of 20 µl. Thereby, 30 PCR cycles were performed in 20-30 minutes at a ramping rate of 6° C per second. Surprisingly, although the average thickness of the walls was 20 microns and the volume of the wells was 35 µl, samples of a volume of as few as 0.5 µl can be easily amplified without reducing the PCR efficiency.

In conclusion, the ultrathin walled multiwell plates according to the invention, allow a simple and rapid loading of multiple samples by conventional pipettes, rapid sealing of all samples by using conventional sealing films and rapid DNA amplification (15-30 minutes for 30 cycles) with an improved specificity typical for rapid cycling (Wittwer et al., Analytical Biochem., 186, 328-331 [1990]) using appropriate heat block thermocyclers (i.e. ramping rate in the range of 4° C to 8° C per second).

Claims

1. Ultrathin-walled multiwell plate for heat block thermocycling of samples comprising an array of small-volume wells of identical height with the similarly shaped sample wells formed in the top surface of the heat block of the thermocycler.

2. Ultrathin-walled multiwell plate according to claim 1, wherein the height of the wells of the plate is not more than the height of the sample wells formed in the top surface of the heat block of the thermocycler

3. Ultrathin-walled multiwell plate according to claim 1, wherein the walls of the wells are conically shaped.

4. Ultrathin-walled multiwell plate according to claim 1, wherein the thickness of the walls of the wells decreases from top to bottom.

5. Ultrathin-walled multiwell plate according to claim 1, wherein the wells of said multiwell plate are thermoformed into negative mould.

6. Ultrathin-walled multiwell plate according to claim 1, wherein the walls of the wells have an average thickness of 20-40 microns.

7. Ultrathin-walled multiwell plate according to claim 1, wherein the walls of the wells are deformable.

8. Ultrathin-walled multiwell plate according to claim 1, wherein the said microwell plate comprises a rigid supporting frame.

9. Ultrathin-walled multiwell plate according to claim 1, wherein the volume of the well is in the range of 16-85 µl.

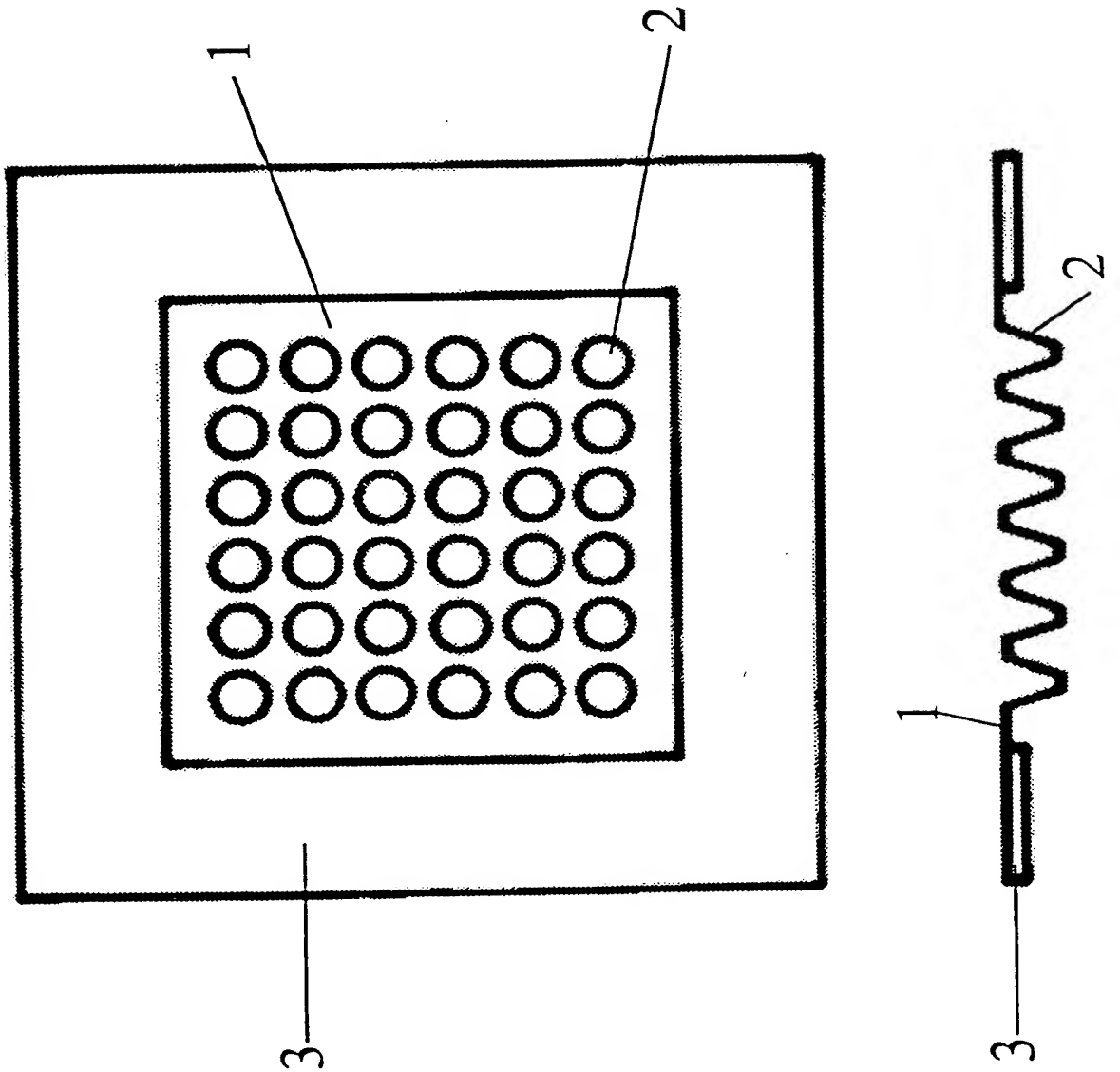

Fig. 1

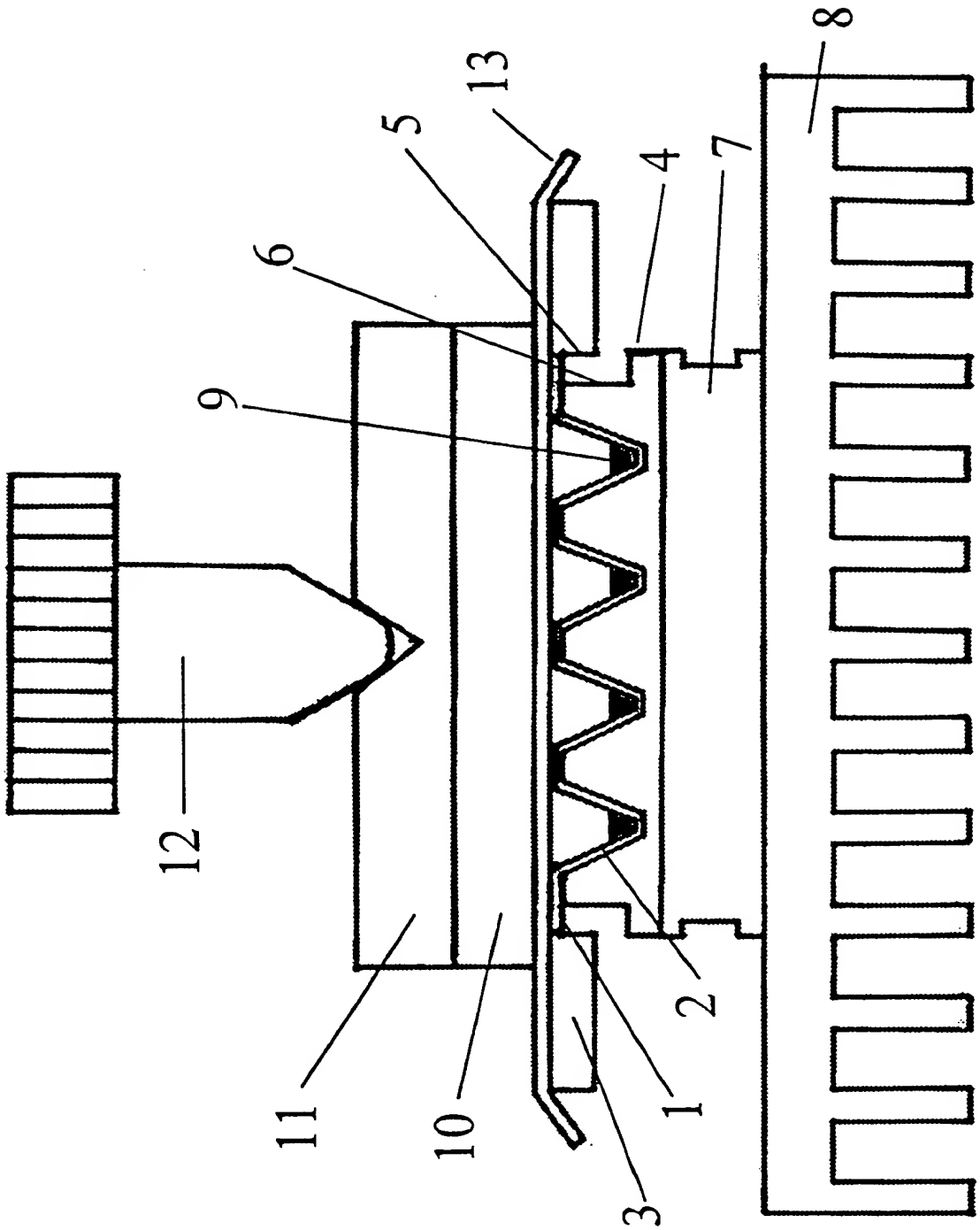

Fig. 2

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 B01L7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 40 22 792 A (MAX PLANCK GESELLSCHAFT) 6 February 1992 (1992-02-06) column 1, line 3 -column 1, line 17 column 1, line 31 -column 1, line 40 column 1, line 46 -column 1, line 52 column 1, line 64 -column 1, line 67 column 2, line 37 -column 2, line 50 column 2, line 65 -column 3, line 6 column 3, line 34 -column 3, line 67 column 4, line 15 -column 4, line 18	1,2,5, 7-9
A	column 4, line 29 -column 4, line 40 column 4, line 58 -column 4, line 68 column 7, line 8 -column 7, line 37	6
A	column 7, line 57 -column 7, line 62 column 16, line 56 -column 17, line 23 figures 1,2,10	4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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17 January 2000	26/01/2000

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 430 957 A (EIGEN MANFRED ET AL) 11 July 1995 (1995-07-11) column 1, line 28 -column 1, line 37 column 4, line 50 -column 4, line 53 column 4, line 62 -column 5, line 2 column 9, line 58 -column 9, line 64 column 10, line 62 -column 11, line 5 figure 5	1,2,8
X	US 5 601 141 A (GORDON STEVEN J ET AL) 11 February 1997 (1997-02-11) column 3, line 31 -column 3, line 45 column 3, line 67 -column 4, line 34 figures 1-4	1,8
X	WO 97 26993 A (BJS COMPANY LTD ;GUNTER IAN ALAN (GB)) 31 July 1997 (1997-07-31) page 1, line 23 -page 2, line 13 page 2, line 19 -page 2, line 24 page 4, line 11 -page 4, line 24 page 5, line 13 -page 5, line 16 figures 1,2	1-3,6,8
A	US 5 161 609 A (MARCADET-TROTON AGNES ET AL) 10 November 1992 (1992-11-10) column 1, line 12 -column 1, line 28 column 4, line 12 -column 4, line 25 column 5, line 64 -column 6, line 3 column 6, line 38 -column 6, line 68 figures 2,4	1,2

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INTERNATIONAL SEARCH REPORT

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PCT/EP 99/08178

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 4022792 A	06-02-1992	AT 125732 T	15-08-1995
		WO 9201513 A	06-02-1992
		DE 59106171 D	07-09-1995
		EP 0539369 A	05-05-1993
		JP 6500727 T	27-01-1994
US 5430957 A	11-07-1995	DE 4029004 C	02-04-1992
		AT 109382 T	15-08-1994
		WO 9204979 A	02-04-1992
		DE 59102453 D	08-09-1994
		EP 0548118 A	30-06-1993
		JP 6502580 T	24-03-1994
US 5601141 A	11-02-1997	NONE	
WO 9726993 A	31-07-1997	AU 1451097 A	20-08-1997
		EP 0876218 A	11-11-1998
US 5161609 A	10-11-1992	FR 2642156 A	27-07-1990
		AT 103062 T	15-04-1994
		AU 4963190 A	13-08-1990
		CA 2025465 A	21-07-1990
		DE 69007305 D	21-04-1994
		DE 69007305 T	29-09-1994
		EP 0379437 A	25-07-1990
		ES 2053128 T	16-07-1994
		WO 9008298 A	26-07-1990
		JP 3503445 T	01-08-1991

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PCT 99/02		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/08178	28/10/1999	29/10/1998
International Patent Classification (IPC) or national classification and IPC B01L3/00		
Applicant HANS-KNÖLL-INSTITUT FÜR NATURSTOFF-FORSCHUNG E.V.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

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3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
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   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
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   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/04/2000	19. 01. 01
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Smith-Hewitt, L Telephone No. +49 89 2399 2995

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08178

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-7	as originally filed				

Claims, No.:

1-8	as received on	10/11/2000	with letter of	08/11/2000	

Drawings, sheets:

1/2,2/2	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-8
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-8
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Re: Item VIII

1.1 The wording of independent claim 1, i.e. 'Ultrathin-walled multiwell plate .... **with the** similarly shaped sample wells formed in the top surface of the heat block of the thermocycler' is such that it is not clear as to whether solely a multiwell plate is being claimed or a multiwell plate **with** a thermocycler. The formation of sample wells in the top surface of the heat block of the thermocycler is clearly not a technical feature of the multi-well plate. It is therefore unclear exactly what is being claimed (the plate or the thermocycler). The requirements of Article 6 PCT with respect to clarity are therefore not fulfilled.

For the purposes of this report, claim 1 has been interpreted as a claim which seeks protection for the multiwell plate only.

1.2 Claim 1 appears to lack the essential feature that the multiwell plate is manufactured from plastic, allowing any multiwell plate and even those manufactured from, for example, silver to be read onto the scope of the claim. As it is clear that the subject-matter of the application applies to plastic plates only, the requirements of Article 6 PCT, when taken in combination with Rule 6.3(a) are not fulfilled.

1.3 Dependent claim 5 attempts to define the device claimed in terms of a method step ('thermoformed') instead of device features, which leads to a lack of clarity in the category of independent claim 1 (Article 6 PCT).

1.4 Claim 8 appears not to be supported by the description (Article 6 PCT). The well volume range tested in the application lies between 16 and 35µl (p.6-7), whereas the claim states a range of 16 to 85µl.

2. Re: Item V

2.1 Document D1 (DE-A-40.22.792), cited by the applicant discloses an ultra-thin walled (c.f. preferred wall thickness <0.08mm, col.4, l.39-40) multiwell plate (2) which **is suitable for** application in the heat block thermocycling of samples, and in particular with PCR reactions (col.17, l.48-66), comprising an array of small volume wells (11) of identical height. These wells fit into similarly shaped wells (102) formed in the top surface of a heat block (106) (cf. col.3, l.56-67, col.6, l.15-18 and col. 16-17 bridging paragraph), such that the outer surface of the plate wells lie in direct contact with the inner walls of the corresponding block wells.

2.2 The subject matter of claim 1 differs from the prior art of D1 only in that it specifically discloses a wall thickness range of between 20 and 40 microns.

2.3 The skilled person wishing to carry out PCR tests using the multiwell plastic tray of D1 would know that polypropylene is a recommended PCR compatible material and is widely used in the art. The same person would also be aware that the physical characteristics of a multiwell tray according to D1 should fulfil the equation cited in claim 1 of D1. Knowing the area and volume of the wells to be used (based on reaction volumes), and the thermal conductivity of the material of the wells selected (in this case polypropylene), the thickness of well required is immediately and obviously derived. Moreover, the specific embodiment of the present application (p.6-7) appears to fall within the bounds of this equation. **Claim 1 cannot therefore be considered to comprise an inventive step in the sense of Article 33(3) PCT.**

2.4 As may be clearly seen from Fig. 10, the height of the wells of the plate (interpreted as the vertical distance between the inner bottom of the plate well and the uppermost surface of the plate film) is not more than the height of the sample wells formed in the top surface of the heat block (interpreted as the vertical distance between the inner bottom of the well and the uppermost surface of the block). **Dependent claim 2 does not, therefore, appear to comprise any additional subject matter which distinguishes the present application from the prior art.** Claim 2 is therefore not inventive, since claim 1 is not inventive (Article 33(3) PCT).

2.5 Given that use of conically shaped wells was well known before the date of filing, **the additional feature of claim 3 does not seem to involve an inventive step in the sense of Article 33(3) PCT.**

2.6 The feature of a gradual reduction in thickness of the walls of the wells from the top to the bottom of the wells is anticipated by document D1. The method of manufacture disclosed therein (Fig. 3) automatically leads to a gradient in the thickness of the wall, whereby the bottom of the well (Fig. 2, 31) is thinner than the side walls (Fig. 2, 32). In other words, a multiwell device formed by vacuumforming plastic material into appropriately shaped wells does not appear to exhibit different physical characteristics to a multiwell plate formed by pushing the same plastic material into appropriately shaped wells with a jet of hot air (both types of well are formed due to a pressure difference between the upper and lower sides of the plastic film). **Dependent claim 4 does not, therefore, appear to comprise any additional subject matter which distinguishes the present application from the prior art.** Claim 4 is therefore not inventive, since claim 1 is not inventive (Article 33(3) PCT).

2.7 In the specific embodiment described in D1, the plastic multi-well plate is manufactured by thermally forming (Fig.3 and col.9, l.3-59) the plastic into a negative mould (i.e. the block 37), although any suitable fast and cheap method of thermal forming (col.3, l.40-46) may be used to produce the plate defined by the features described in column 2. The walls of the wells are therefore 'deformable', i.e. may be deformed e.g. by heating. **Dependent claims 5 and 6 do not, therefore, appear to comprise any additional subject matter which distinguishes the present application from the prior art.** Claims 5 and 6 are therefore not inventive, since claim 1 is not inventive (Article 33(3) PCT).

2.8 **The additional feature of the rigid supporting frame in claim 7 does not seem to be inventive in the sense of Article 33(3) PCT.**

2.9 The well volume preferably used in D1 is between 10 and 100µl (col.4, l.17). As the selection of the range 16-85µl in claim 8 of the present application does not seem to be based on a special technical effect, but rather appears to be based upon the typical volumes used especially in PCR reactions, **the additional**

subject matter described in Claim 8 cannot be considered as comprising an inventive step in the sense of Article 33(3) PCT.

3. Re: Item VII

3.1 Independent claim 1 is not in the two-part form in accordance with Rule 6.3(b) PCT.

3.2 The features of the claims are not provided with reference signs placed in parentheses (Rule 6.2(b) PCT).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PCT 99/02		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/08178	28/10/1999	29/10/1998
Applicant		
HANS-KNÖLL-INSTITUT FÜR NATURSTOFF-FORSCHUNG E.V.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 99/08178

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 B01L7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 40 22 792 A (MAX PLANCK GESELLSCHAFT) 6 February 1992 (1992-02-06) column 1, line 3 -column 1, line 17 column 1, line 31 -column 1, line 40 column 1, line 46 -column 1, line 52 column 1, line 64 -column 1, line 67 column 2, line 37 -column 2, line 50 column 2, line 65 -column 3, line 6 column 3, line 34 -column 3, line 67 column 4, line 15 -column 4, line 18	1,2,5, 7-9
A	column 4, line 29 -column 4, line 40 column 4, line 58 -column 4, line 68 column 7, line 8 -column 7, line 37	6
A	column 7, line 57 -column 7, line 62 column 16, line 56 -column 17, line 23 figures 1,2,10	4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 January 2000	26/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Koch, A

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 99/08178

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 430 957 A (EIGEN MANFRED ET AL) 11 July 1995 (1995-07-11) column 1, line 28 -column 1, line 37 column 4, line 50 -column 4, line 53 column 4, line 62 -column 5, line 2 column 9, line 58 -column 9, line 64 column 10, line 62 -column 11, line 5 figure 5	1,2,8
X	US 5 601 141 A (GORDON STEVEN J ET AL) 11 February 1997 (1997-02-11) column 3, line 31 -column 3, line 45 column 3, line 67 -column 4, line 34 figures 1-4	1,8
X	WO 97 26993 A (BJS COMPANY LTD ;GUNTER IAN ALAN (GB)) 31 July 1997 (1997-07-31) page 1, line 23 -page 2, line 13 page 2, line 19 -page 2, line 24 page 4, line 11 -page 4, line 24 page 5, line 13 -page 5, line 16 figures 1,2	1-3,6,8
A	US 5 161 609 A (MARCADET-TROTON AGNES ET AL) 10 November 1992 (1992-11-10) column 1, line 12 -column 1, line 28 column 4, line 12 -column 4, line 25 column 5, line 64 -column 6, line 3 column 6, line 38 -column 6, line 68 figures 2,4	1,2

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/08178

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 4022792 A	06-02-1992	AT 125732 T	15-08-1995
		WO 9201513 A	06-02-1992
		DE 59106171 D	07-09-1995
		EP 0539369 A	05-05-1993
		JP 6500727 T	27-01-1994
US 5430957 A	11-07-1995	DE 4029004 C	02-04-1992
		AT 109382 T	15-08-1994
		WO 9204979 A	02-04-1992
		DE 59102453 D	08-09-1994
		EP 0548118 A	30-06-1993
		JP 6502580 T	24-03-1994
US 5601141 A	11-02-1997	NONE	
WO 9726993 A	31-07-1997	AU 1451097 A	20-08-1997
		EP 0876218 A	11-11-1998
US 5161609 A	10-11-1992	FR 2642156 A	27-07-1990
		AT 103062 T	15-04-1994
		AU 4963190 A	13-08-1990
		CA 2025465 A	21-07-1990
		DE 69007305 D	21-04-1994
		DE 69007305 T	29-09-1994
		EP 0379437 A	25-07-1990
		ES 2053128 T	16-07-1994
		WO 9008298 A	26-07-1990
		JP 3503445 T	01-08-1991

M-H

VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS

PCT

INTERNATIONALER RECHERCHENBERICHT

(Artikel 18 sowie Regeln 43 und 44 PCT)

Aktenzeichen des Anmelders oder Anwalts	WEITERES VORGEHEN	siehe Mitteilung über die Übermittlung des internationalen Recherchenberichts (Formblatt PCT/ISA/220) sowie, soweit zutreffend, nachstehender Punkt 5
PCT 99/02		
Internationales Aktenzeichen	Internationales Anmeldedatum *(Tag/Monat/Jahr)*	(Frühestes) Prioritätsdatum *(Tag/Monat/Jahr)*
PCT/EP 99/08178	28/10/1999	29/10/1998

Anmelder

HANS-KNÖLL-INSTITUT FÜR NATURSTOFF-FORSCHUNG E.V.

Dieser internationale Recherchenbericht wurde von der Internationalen Recherchenbehörde erstellt und wird dem Anmelder gemäß Artikel 18 übermittelt. Eine Kopie wird dem Internationalen Büro übermittelt.

Dieser internationale Recherchenbericht umfaßt insgesamt 3 Blätter.

[X] Darüber hinaus liegt ihm jeweils eine Kopie der in diesem Bericht genannten Unterlagen zum Stand der Technik bei.

1. **Grundlage des Berichts**

   a. Hinsichtlich der **Sprache** ist die internationale Recherche auf der Grundlage der internationalen Anmeldung in der Sprache durchgeführt worden, in der sie eingereicht wurde, sofern unter diesem Punkt nichts anderes angegeben ist.

   [ ] Die internationale Recherche ist auf der Grundlage einer bei der Behörde eingereichten Übersetzung der internationalen Anmeldung (Regel 23.1 b)) durchgeführt worden.

   b. Hinsichtlich der in der internationalen Anmeldung offenbarten **Nucleotid- und/oder Aminosäuresequenz** ist die internationale Recherche auf der Grundlage des Sequenzprotokolls durchgeführt worden, das

   [ ] in der internationalen Anmeldung in Schriftlicher Form enthalten ist.

   [ ] zusammen mit der internationalen Anmeldung in computerlesbarer Form eingereicht worden ist.

   [ ] bei der Behörde nachträglich in schriftlicher Form eingereicht worden ist.

   [ ] bei der Behörde nachträglich in computerlesbarer Form eingereicht worden ist.

   [ ] Die Erklärung, daß das nachträglich eingereichte schriftliche Sequenzprotokoll nicht über den Offenbarungsgehalt der internationalen Anmeldung im Anmeldezeitpunkt hinausgeht, wurde vorgelegt.

   [ ] Die Erklärung, daß die in computerlesbarer Form erfaßten Informationen dem schriftlichen Sequenzprotokoll entsprechen, wurde vorgelegt.

2. [ ] **Bestimmte Ansprüche haben sich als nicht recherchierbar erwiesen** (siehe Feld I).

3. [ ] **Mangelnde Einheitlichkeit der Erfindung** (siehe Feld II).

4. Hinsichtlich der **Bezeichnung der Erfindung**

   [X] wird der vom Anmelder eingereichte Wortlaut genehmigt.

   [ ] wurde der Wortlaut von der Behörde wie folgt festgesetzt:

5. Hinsichtlich der **Zusammenfassung**

   [X] wird der vom Anmelder eingereichte Wortlaut genehmigt.

   [ ] wurde der Wortlaut nach Regel 38.2b) in der in Feld III angegebenen Fassung von der Behörde festgesetzt. Der Anmelder kann der Behörde innerhalb eines Monats nach dem Datum der Absendung dieses internationalen Recherchenberichts eine Stellungnahme vorlegen.

6. Folgende Abbildung der **Zeichnungen** ist mit der Zusammenfassung zu veröffentlichen: Abb. Nr. 2

   [ ] wie vom Anmelder vorgeschlagen

   [ ] keine der Abb.

   [X] weil der Anmelder selbst keine Abbildung vorgeschlagen hat.

   [ ] weil diese Abbildung die Erfindung besser kennzeichnet.

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
PCT/[redacted]99/08178

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 7 B01L3/00 B01L7/00

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
IPK 7 B01L

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie°	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	DE 40 22 792 A (MAX PLANCK GESELLSCHAFT) 6. Februar 1992 (1992-02-06) Spalte 1, Zeile 3 -Spalte 1, Zeile 17 Spalte 1, Zeile 31 -Spalte 1, Zeile 40 Spalte 1, Zeile 46 -Spalte 1, Zeile 52 Spalte 1, Zeile 64 -Spalte 1, Zeile 67 Spalte 2, Zeile 37 -Spalte 2, Zeile 50 Spalte 2, Zeile 65 -Spalte 3, Zeile 6 Spalte 3, Zeile 34 -Spalte 3, Zeile 67 Spalte 4, Zeile 15 -Spalte 4, Zeile 18	1,2,5, 7-9
A	Spalte 4, Zeile 29 -Spalte 4, Zeile 40 Spalte 4, Zeile 58 -Spalte 4, Zeile 68 Spalte 7, Zeile 8 -Spalte 7, Zeile 37	6
A	Spalte 7, Zeile 57 -Spalte 7, Zeile 62 Spalte 16, Zeile 56 -Spalte 17, Zeile 23 Abbildungen 1,2,10	4

-/--

[X] Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

[X] Siehe Anhang Patentfamilie

° Besondere Kategorien von angegebenen Veröffentlichungen :

"A" Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist

"E" älteres Dokument, das jedoch erst am oder nach dem internationalen Anmeldedatum veröffentlicht worden ist

"L" Veröffentlichung, die geeignet ist, einen Prioritätsanspruch zweifelhaft erscheinen zu lassen, oder durch die das Veröffentlichungsdatum einer anderen im Recherchenbericht genannten Veröffentlichung belegt werden soll oder die aus einem anderen besonderen Grund angegeben ist (wie ausgeführt)

"O" Veröffentlichung, die sich auf eine mündliche Offenbarung, eine Benutzung, eine Ausstellung oder andere Maßnahmen bezieht

"P" Veröffentlichung, die vor dem internationalen Anmeldedatum, aber nach dem beanspruchten Prioritätsdatum veröffentlicht worden ist

"T" Spätere Veröffentlichung, die nach dem internationalen Anmeldedatum oder dem Prioritätsdatum veröffentlicht worden ist und mit der Anmeldung nicht kollidiert, sondern nur zum Verständnis des der Erfindung zugrundeliegenden Prinzips oder der ihr zugrundeliegenden Theorie angegeben ist

"X" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann allein aufgrund dieser Veröffentlichung nicht als neu oder auf erfinderischer Tätigkeit beruhend betrachtet werden

"Y" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als auf erfinderischer Tätigkeit beruhend betrachtet werden, wenn die Veröffentlichung mit einer oder mehreren anderen Veröffentlichungen dieser Kategorie in Verbindung gebracht wird und diese Verbindung für einen Fachmann naheliegend ist

"&" Veröffentlichung, die Mitglied derselben Patentfamilie ist

Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
14. Januar 2000	20/01/2000

Name und Postanschrift der Internationalen Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Koch, A

1

Formblatt PCT/ISA/210 (Blatt 2) (Juli 1992)

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
PCT/[redacted]99/08178

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie°	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	US 5 430 957 A (EIGEN MANFRED ET AL) 11. Juli 1995 (1995-07-11) Spalte 1, Zeile 28 -Spalte 1, Zeile 37 Spalte 4, Zeile 50 -Spalte 4, Zeile 53 Spalte 4, Zeile 62 -Spalte 5, Zeile 2 Spalte 9, Zeile 58 -Spalte 9, Zeile 64 Spalte 10, Zeile 62 -Spalte 11, Zeile 5 Abbildung 5	1,2,8
X	US 5 601 141 A (GORDON STEVEN J ET AL) 11. Februar 1997 (1997-02-11) Spalte 3, Zeile 31 -Spalte 3, Zeile 45 Spalte 3, Zeile 67 -Spalte 4, Zeile 34 Abbildungen 1-4	1,8
X	WO 97 26993 A (BJS COMPANY LTD ;GUNTER IAN ALAN (GB)) 31. Juli 1997 (1997-07-31) Seite 1, Zeile 23 -Seite 2, Zeile 13 Seite 2, Zeile 19 -Seite 2, Zeile 24 Seite 4, Zeile 11 -Seite 4, Zeile 24 Seite 5, Zeile 13 -Seite 5, Zeile 16 Abbildungen 1,2	1-3,6,8
A	US 5 161 609 A (MARCADET-TROTON AGNES ET AL) 10. November 1992 (1992-11-10) Spalte 1, Zeile 12 -Spalte 1, Zeile 28 Spalte 4, Zeile 12 -Spalte 4, Zeile 25 Spalte 5, Zeile 64 -Spalte 6, Zeile 3 Spalte 6, Zeile 38 -Spalte 6, Zeile 68 Abbildungen 2,4	1,2

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/[redacted] 99/08178

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
DE 4022792	A	06-02-1992	AT	125732 T	15-08-1995
			WO	9201513 A	06-02-1992
			DE	59106171 D	07-09-1995
			EP	0539369 A	05-05-1993
			JP	6500727 T	27-01-1994
US 5430957	A	11-07-1995	DE	4029004 C	02-04-1992
			AT	109382 T	15-08-1994
			WO	9204979 A	02-04-1992
			DE	59102453 D	08-09-1994
			EP	0548118 A	30-06-1993
			JP	6502580 T	24-03-1994
US 5601141	A	11-02-1997	NONE		
WO 9726993	A	31-07-1997	AU	1451097 A	20-08-1997
			EP	0876218 A	11-11-1998
US 5161609	A	10-11-1992	FR	2642156 A	27-07-1990
			AT	103062 T	15-04-1994
			AU	4963190 A	13-08-1990
			CA	2025465 A	21-07-1990
			DE	69007305 D	21-04-1994
			DE	69007305 T	29-09-1994
			EP	0379437 A	25-07-1990
			ES	2053128 T	16-07-1994
			WO	9008298 A	26-07-1990
			JP	3503445 T	01-08-1991

PA[illegible]NT COOPERATION TREAT[illegible]

From the INTERNATIONAL BUREAU

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
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in its capacity as elected Office

Date of mailing (day/month/year) 02 June 2000 (02.06.00)	
International application No. PCT/EP99/08178	**Applicant's or agent's file reference** PCT 99/02
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Applicant TRETIAKOV, Alexandre et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

29 April 2000 (29.04.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
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